Published online May 7, 2007

Discerning and Modeling the Fate and Transport of Testosterone in Undisturbed Soil

Zhaosheng Fan, Francis X. M. Casey,* Heldur Hakk, and Gerald L. Larsen

ABSTRACT

Testosterone is an endocrine disruptor that is released into the environment from natural and anthropogenic sources. The objective of this study was to achieve a better understanding of the complex fate and transport of this labile compound in an undisturbed agricultural soil (a Hamar Sandy, mixed, frigid typic Endoaquolls). This was done by using batch and miscible-displacement experiments, and by using a chemical nonequilibrium transport model. Sorption and transformations of testosterone were discerned using various batch experiments. The batch experiments indicated that the aqueous phase concentrations of testosterone rapidly decreased from 12 to 15% of the initial aqueous concentration within 5 h, but then gradually increased through time and reached 28 to 29% of the initial aqueous concentration at 168 h. The increase in the aqueous concentration was explained by mineralization and biodegradation. Multiple first-order models were used to describe batch experiments where simultaneous degradation and sorption processes occurred. An evolutionary global optimization strategy was used to estimate the process parameters from these batch experiments and there was high confidence in these parameter estimates. The result of column experiments also showed that 23.4% of testosterone was mineralized to CO_2 as it transported through the column. Combustion analyses of extracted soil from inside the columns showed that most of the ^{14}C retained in the column (69–74%) was sorbed in the top 5 cm. The independently determined batch parameters were incorporated into a chemical nonequilibrium transport model, which provided an excellent description of the hormone in the effluent, and vertical redistribution in the soil column.

In recent years, the detection of testosterone and other potent reproductive hormones (e.g., 17β-estradiol) in the environment has drawn increasing concern among scientists, regulators, and citizens. Several studies have shown that extended exposure to low concentrations of some sex hormones can alter the endocrine and reproductive systems of animals (Panter et al., 1998; Larsson et al., 2000; Iguchi et al., 2001). For example, Koger et al. (2000) exposed medaka (*Oryzias latipes*) to 100 μg L^{-1} of testosterone for 6 d and found that newly hatched fry or 1 wk post-hatch fish displayed intersex gonads. The majority of reproductive hormones in the environment come from human and animal sources (Shore and Shemesh, 2003). A survey of 139 U.S. streams in 1999 and 2000 showed that reproductive hormones were detected at frequencies and concentrations that may have significant implications on the health of aquatic organisms (Kolpin et al., 2002). These reproductive hormone detections may have greater implications for testosterone, which tends to have more mobility in soils than estrogenic hormones (Casey et al., 2004).

Casey et al. (2004) used several batch and column experiments to study the sorption, mobility, and transformation of testosterone in agricultural soils. Their batch experiments indicate that testosterone sorption is correlated to soil particle size, organic matter, and specific surface area. The range of log K_{oc} values from these batch experiments was 2.97 to 4.56. Casey et al. (2004) used a convective-dispersive transport model with degradation in the sorbed phase and fully kinetic Freundlich sorption to describe their soil column experiments. They estimated first-order testosterone degradation rates of 0.40 to 0.60 h^{-1} and kinetic sorption rates of 0.1 to 0.6 h^{-1}. During groundwater recharge via soil aquifer treatment, Mansell et al. (2004) reported that the major removal mechanism of testosterone is biodegradation and sorption. Mansell et al. (2004) indicated that biodegradation of testosterone is independent of soil type or soil organic carbon (OC). In soil batch experiments, Lee et al. (2003) measured testosterone log K_{oc} values that ranged from 3.25 to 3.52 and first-order half-lives that ranged from 0.012 to 0.304 h^{-1}. Lee et al. (2003) also indicates that OC was the main sorption domain for testosterone. Das et al. (2004) modeled testosterone column breakthrough curves using a similar convective-dispersive equation as Casey et al. (2004), but described sorption as a partially kinetic process (not fully kinetic) with a linear isotherm. Das et al. (2004) used log K_{oc} values from the Lee et al. (2003) study to model their column data and estimated kinetic sorption rate values that ranged from 0.49 to 0.87 h^{-1}. Das et al. (2004) also assumed degradation occurred on the sorbed phase and estimate degradation rates to be 0.002 to 0.015 h^{-1}.

These initial studies have identified some sorption and degradation processes of testosterone in soil; however, the fate and transport of this chemical in the environment is still not fully understood. Three major weaknesses of previous studies include (i) an inappropriate assumption that testosterone degradation occurs on the sorbed phase, (ii) a failure to fully quantify testosterone biotransformation by not trapping CO_2 released from soil experiments, and (iii) inaccuracies associated with correctly identifying unique fate and transport parameter estimates. The objective of this research was to investigate the fate and transport processes of testosterone more completely in undisturbed soil. This was done by using improved batch and miscible-displacement experiments (e.g., where CO_2 is trapped), and by using a comprehensive transport model with improved parameter estimation capabilities. The unique combination of detailed experiments with model development improves

Z. Fan and F.X.M. Casey, Dep. of Soil Science, North Dakota State Univ., Fargo, ND 58105. H. Hakk and G.L. Larsen, USDA-ARS, Animal Metabolism-Agricultural Chemicals Research, Biosciences Research Lab., Fargo, ND 58105. Received 16 Oct. 2006. *Corresponding author (francis.casey@ndsu.edu).

Published in J. Environ. Qual. 36:864–873 (2007).
Technical Reports: Vadose Zone Processes and Chemical Transport
doi:10.2134/jeq2006.0451

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: LO, local optimization; OC, organic carbon; ODE, ordinary differential equation; SRES, stochastic ranking evolutionary strategy; SSQ, sum-of-squares residuals; TLC, thin-layer chromatography.

the means to identify coupled biological, physical, and chemical processes in soil for testosterone and other labile compounds.

MATERIALS AND METHODS

Soil Collection

Samples of a Hamar (Sandy, mixed, frigid typic Endoaquolls) soil were collected using pre-cleaned stainless steel cylinders (i.d. 15 cm, height 30 cm). The cylinders were beveled at one end and a specialized drop hammer was used to drive the beveled end into the soil surface. Before the soil cores were taken all the surface groundcover was removed. Once the cylinder was driven fully into the soil, it was then excavated with a shovel, and capped with grooved (to fit the cylinder diameter) Teflon plates. As a result the soil structure was not disturbed and remained identical to those under field conditions. The following soil physical properties were determined: bulk density of 1.54 g cm^{-3}, porosity of 0.42, organic matter of 2.23%, 14.0% clay, 19.0% silt, and 67% sand.

Chemicals

[4-^{14}C]-Radiolabeled testosterone (>99% by thin-layer chromatography [TLC]) was purchased from the American Radiolabeled Chemicals, St. Louis, MO. Testosterone has a molecular weight of 288.4 g mol^{-1}, a water solubility of 5.57 mg L^{-1} at 25°C, and a $\log_{10}$ octanol-water partition coefficient ($\log K_{ow}$) of 3.32 (Lintelmann et al., 2003).

Batch Sorption Experiments

A series of batch experiments were conducted in the laboratory to determine the sorption of testosterone in natural soils. Batch experiments were all run in triplicate and at room temperature (21 ± 1°C).

Sieved (2 mm) Hamar soil and a weak salt solution, 0.01 *M* $CaCl_2$ (Sigma-Aldrich, St. Louis, MO), were added to 10-mL clear vials sealed with silicon/Teflon septa (Microliter Analytical Supplies, Inc., Suwanee, GA) in a ratio of 1.6 g of soil to 8 mL of 0.01 *M* $CaCl_2$. [4-^{14}C]-Radiolabeled testosterone was then added to these vials to create solution concentrations of 0.406 and 0.738 mg L^{-1}. These concentrations were chosen because they have been found in animal manures applied to agricultural fields (Shore and Shemesh, 2003; Lorenzen et al., 2004). These soil-water slurries were then agitated by rotating the vials from top to bottom (360° every 5 s). After 0.5, 1, 5, 24, 48, and 168 h, the bottles were centrifuged at 1700 rpm (380 × *g* for 20 min), and triplicate 100-μL aliquots were withdrawn through the septa of these vials using a sterile syringe. These samples were then assayed for radioactivity by liquid scintillation counting using a 1900 CA scintillation counter (Packard, Downers Grove, IL).

To examine the possibility of mineralization and photodegradation in the natural soils during the batch experiments, another two sets of controlled batch experiments were conducted in clear and amber vials with sterile soil. The sterile soil was prepared in the following order: (i) adding 1.6 g of air-dried Hamar soil to a vial, (ii) sealing the vial with silicon/Teflon septa cap, (iii) autoclaving the soil and vial with cap in place for 40 min at 122°C (a syringe was inserted through the septa to release air pressure during autoclaving), and (iv) adding the prepared [4-^{14}C]-radiolabeled testosterone stock solution with 8 mL of 0.01 *M* $CaCl_2$ to the vial using a sterile syringe and needle. During the whole process, the cap was not removed to ensure sterile conditions. The initial concentrations of testosterone and the experimental procedure were the same as batch experiments discussed earlier.

Batch Sorption and Biodegradation Model

Previous studies have shown that sorption of hormones are described efficiently with linear sorption isotherms (Lee et al., 2003; Das et al., 2004). To describe the sorption and biodegradation of testosterone in the natural soils of this study, linear isotherms with degradation and mineralization in the aqueous phase were used. Figure 1 and the following set of first-order differential equations describe the processes that occur in the batch experiments:

$$\begin{cases} \dfrac{\partial S_1}{\partial t} = \alpha_1(K_{d,1}C_1 - S_1) \\ \dfrac{\partial C_1}{\partial t} = -\dfrac{M}{V}\alpha_1(K_{d,1}C_1 - S_1) - \omega_{w,1}C_1 - \omega_{m,1}C_1 \\ \dfrac{\partial (CO_2)_1}{\partial t} = \omega_{m,1}C_1 \end{cases} \quad [1]$$

$$\begin{cases} \dfrac{\partial S_2}{\partial t} = \alpha_2(K_{d,2}C_2 - S_2) \\ \dfrac{\partial C_2}{\partial t} = -\dfrac{M}{V}\alpha_2(K_{d,2}C_2 - S_2) + \omega_{w,1}C_1 - \omega_{m,2}C_2 \\ \dfrac{\partial (CO_2)_2}{\partial t} = \omega_{m,2}C_2 \end{cases} \quad [2]$$

$$\frac{\partial [C(^{14}C)]}{\partial t} = \frac{\partial C_1}{\partial t} + \frac{\partial (CO_2)_1}{\partial t} + \frac{\partial C_2}{\partial t} + \frac{\partial (CO_2)_2}{\partial t} \quad [3]$$

The mass balance for the batch experiment is given as:

$$\begin{cases} -M\dfrac{\partial S}{\partial t} = V\dfrac{\partial [C(^{14}C)]}{\partial t} \\ S = S_1 + S_2 \end{cases} \quad [4]$$

The subscripts 1 and 2 represent the parent testosterone compound and its metabolite, respectively; C is the aqueous concentration (mg L^{-1}); S is the sorbed phase concentration (mg g^{-1}); K_d (L g^{-1}) is the linear distribution coefficient between the sorbed and aqueous phases; ω_w is the first-order transformation rate constant in the liquid phase (h^{-1}); ω_m is mineralization rate constant of testosterone (h^{-1}); α is the sorption mass transfer coefficient (h^{-1}); $(CO_2)_1$ and $(CO_2)_2$ (mg L^{-1}) represent the CO_2 that was released due to the mineralization of testosterone and its metabolite, respectively; $C(^{14}C)$ (mg L^{-1}) is the total concentration of ^{14}C detected in the liquid phase. Equations [1–3] contain the following seven unknown parameters: $K_{d,1}$, $K_{d,2}$, $\omega_{w,1}$, $\omega_{m,1}$, $\omega_{m,2}$, α_1, and α_2.

Due to the complexity of the nonlinear mathematical models and the large numbers of parameters, models like the one above are frequently ill-conditioned and multimodal (Banga et al., 2003). Thus, traditional local optimization (LO) parameter estimation methods are not suitable to find the absolutely best set of parameters, because LO methods can be easily entrapped in a local minimum. Also, LO methods cannot use global information to seek the global minimum of an objective function that has many local minima. The stochastic ranking evolutionary strategy (SRES) (Runarsson and Yao, 2000), a global optimization method, was used to solve this problem. The SRES uses a "parent and offspring" (μ, λ) evolution strategy coupled with stochastic ranking as the constraint handling technique to obtain the global minimum of a given objective function. The SRES can automatically balance objective and penalty functions stochastically during the evolutionary search (Runarsson and Yao, 2000). Several studies have demonstrated that SRES is more competitive than other global optimization methods

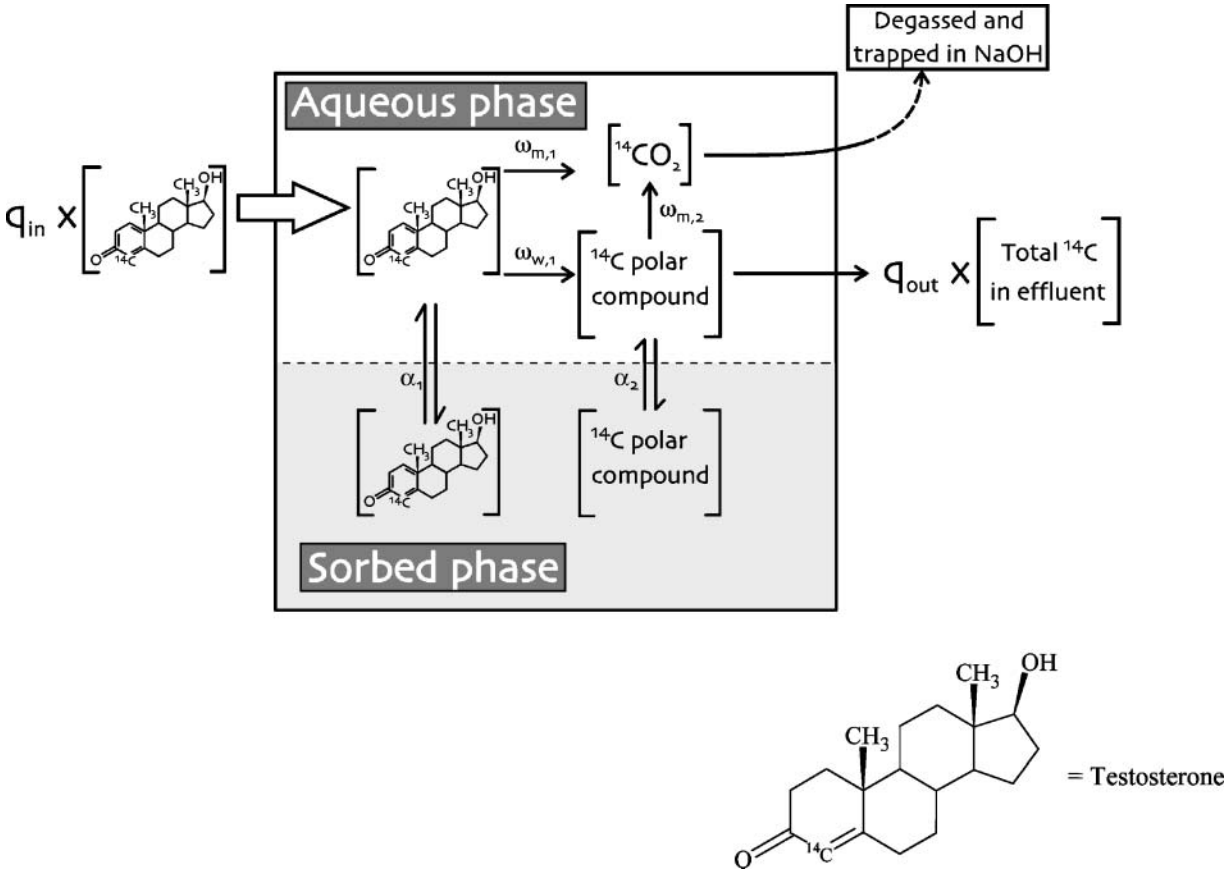

Fig. 1. Schematic of the models that were used to describe the fate and transport of testosterone. The convection processes and gas collection are limited only to miscible-displacement experiments.

to uniquely solve parameter estimation problems with large numbers of local solutions and large dimensionality (Runarsson and Yao, 2000; Banga et al., 2003; Moles et al., 2003). For example, Moles et al. (2003) used SRES to inversely estimate 36 parameters of a biochemical process model, and showed that it outperformed other global optimization methods.

The global optimization problem for the batch experiments was sought to minimize the objective function, *J*, which was defined as

$$J = \sum_{i=1}^{l} \sum_{j=1}^{n} w_{ij} \{[C(^{14}\mathrm{C})_i - \overline{C}(^{14}\mathrm{C})_i]_j\}^2 \qquad [5]$$

and subjected to the following conditions:

$$\begin{cases} 0.01 \le K_{d,1} \le 1.0,\ 0.01 \le K_{d,2} \le 1.0 \\ 0.01 \le \omega_{w,1} \le 10.0 \\ 0.003 \le \omega_{m,1} \le 0.1,\ 0.003 \le \omega_{m,2} \le 0.1 \\ 0.1 \le \alpha_1 \le 1.0,\ 0.0001 \le \alpha_2 \le 0.01 \\ C(t \to 168)_{\text{testosterone}} \to 0.0 \end{cases} \qquad [6]$$

In Eq. [5] and [6], *n* was the number of experiments, *l* was the number of data for each experiment, $C(^{14}\mathrm{C})_i$ (mg L^{-1}) was the experimental concentration of ^{14}C, $\overline{C}(^{14}\mathrm{C})_i$ (mg L^{-1}) was the predicted concentration of ^{14}C using the model with a given set of the seven parameters (Eq. [1–4]), and w_{ij} was a weighting function. For the batch experiments, *k* was set to 2 representing the two different initial concentrations, 0.738 and 0.406 mg L^{-1}; *l* was set to 6 representing the six time points 0.5, 1, 5, 24, 48, and 168 h; and w_{ij} was set to 1 (meaning all the experimental data are treated equally). The parameter bounds were set based on previous studies (Casey et al., 2004; Fan et al., 2007).

A C library, libSRES (Ji and Xu, 2006), was used to solve the SRES problem. To solve the ordinary differential equations (ODE) (i.e., Eqs [1], [2], and [3]), an ODE solver, CVODE (Cohen and Hindmarsh, 1994), was incorporated into libSRES. This ODE solver, CVODE, is suitable for both non-stiff and stiff problems. Also a nonparametric bootstrap method was used to estimate the 95% confidence interval for each parameter (Efron and Tibshirani, 1993).

Miscible-Displacement Experiments

Experiment 1

The miscible-displacement experimental setup is shown in Fig. 2. The soil column was turned upside down so that the flow rate was easily controlled. To minimize the air entrapment, the soil column was slowly wetted (1 mL min^{-1} over 72 h) with a weak salt solution (0.01 *M* $CaCl_2$) using a high-pressure solvent pump. The weak salt solution was used so soil aggregates would not be dispersed. After saturation was achieved, a steady water flow rate of 7 mL min^{-1} was established through the column using the same 0.01 *M* $CaCl_2$ solution. After steady-state water flow was established and maintained, a pulse (about 2 pore volumes) of higher $CaCl_2$ concentrations (0.05 *M* $CaCl_2$) was passed through the column and eluted with the 0.01 *M* $CaCl_2$ solution. The column effluent was fraction collected every 3.5 min, and the conductivity of each fraction was measured using a conductivity meter (Oakton PC 300, Vernon Hills, IL). This 0.05 *M* $CaCl_2$ breakthrough experiment

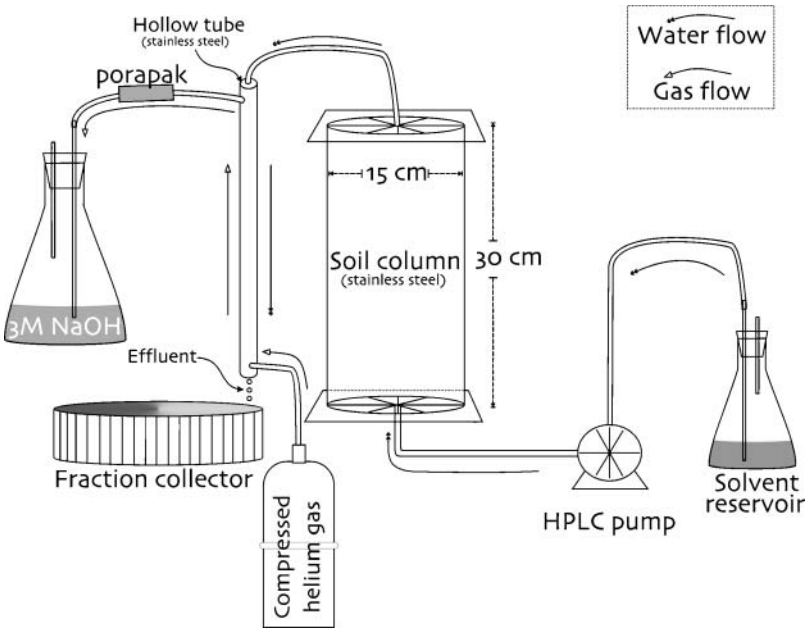

Fig. 2. Schematic of the miscible-displacement experimental setup. Tygon tubing (6-mm i.d.) was used for all connections. In Experiment 1, the effluents from the soil column were directly collected in the fraction collector without running through the stainless steel cylinder and trapping $^{14}CO_2$ in NaOH.

provided information about the physical transport of a nonsorbing solute through this undisturbed Hamar soil.

Following this chloride breakthrough curve experiment, a pulse of [4-^{14}C]-testosterone (approximately 5.9 μCi, 6 000 000 dpm) was applied to the column in 300 mL of 0.01 *M* $CaCl_2$ and eluted with at least 20 L (approximately 6 pore volumes) of 0.01 *M* $CaCl_2$. The column effluent was fraction collected every 3.5 min, and each fraction was analyzed for ^{14}C using liquid scintillation counting described earlier. Thin-layer chromatography [System 2000 Imaging Scanner (Bioscan, Inc., Washington DC)] was also used to determine the presence of any testosterone metabolites in the column effluent.

Additionally, distribution of resident ^{14}C inside the soil column was determined at the end of the experiment. This was done by extruding the soil from the column in 5-cm increments, drying the extruded soil, and assaying it for ^{14}C with combustion analysis using a Packard Model 307 Oxidizer (Meridan, CT). Analysis for metabolites was also done on these extracts using TLC.

Experiment 2

The result of Experiment 1 indicated a low total mass recovery, which was calculated by adding the ^{14}C mass from the effluent and from within the column. It was hypothesized that the mass-balance errors were a result of the continued mineralization of testosterone at the end of the experiment, and the loss of volatile testosterone metabolites from the column effluent. A nearly identical column experiment was repeated with two major modifications to minimize mass loss. First, the column experiment was modified to collect any ^{14}C-labeled volatile compounds in the effluent. To do this the effluent droplets were passed through a stainless steel cylinder (i.d. 2 cm, height 40 cm; Fig. 2). This cylinder directed the effluent to stream down the cylinder wall (without dripping), which increased the air-water contact area. Also, a gas inlet, through which helium gas was blown, was connected at the bottom of this stainless steel cylinder. The helium gas was directed upward through the cylinder to an outlet. The outlet gas was first passed through a Porapak Q column (i.d. 0.5 cm, length 6 cm) (Porapak type Q, 100–200 mesh; Waters Corporation, Milford, MA), which trapped the ^{14}C-labeled volatile compounds. The Porapak was cleaned by washing in the following order: 45 mL of MeOH, 45 mL of acetone (>99%; Fisher Chemical, Fairlawn, NJ), 45 mL of MeOH, and 45 mL of deionized H_2O, and then was air-dried at room temperature. After passing through the Porapak column, the outlet gas continued through 3 *M* NaOH solution, which trapped $^{14}CO_2$. The second major modification to the column experiment was to stop biological activity at the end of the experiment. This was done by applying 1 pore volume of 0.75 mg L^{-1} of $HgCl_2$ to the soil column at the end of experiment, which would inhibit nearly all testosterone biodegradation. All other experimental procedures were the same as Experiment 1.

Column Transport Model

To describe the fate and transport of testosterone, the one-site chemical nonequilibrium transport with steady-state flow and linear sorption was used (van Genuchten, 1981). Also, solutes involved in a chain reaction were considered, where one compound is transformed into another while undergoing convective-dispersive transport (van Genuchten, 1985). This model concept is presented in Fig. 1. The following partial differential equation quantifies this transport model:

$$\begin{cases} \theta\dfrac{\partial C_1}{\partial t} + \rho_b\dfrac{\partial S_1}{\partial t} = \theta v\lambda\dfrac{\partial^2 C_1}{\partial x^2} - \theta v\dfrac{\partial C_1}{\partial x} - (\omega_{m,1} + \omega_{w,1})\theta C_1 \\ \theta\dfrac{\partial C_2}{\partial t} + \rho_b\dfrac{\partial S_2}{\partial t} = \theta v\lambda\dfrac{\partial^2 C_2}{\partial x^2} - \theta v\dfrac{\partial C_2}{\partial x} + \omega_{w,1}\theta C_1 - \omega_{m,2}\theta C_2 \end{cases} \quad [7]$$

where the subscripts 1 and 2 represent the parent solute, testosterone, and the metabolite, respectively; ρ_b is soil bulk density (kg m^{-3}); θ is the volumetric water content (cm^3 cm^{-3}); λ is the dispersivity (cm); *v* is the pore water velocity (cm h^{-1}); *x* is depth (cm); and α, ω_m, ω_w, *C*, and *S* are defined in Eq. [1–3] from the batch experiments.

The initial condition for this problem is given as

$$C(x,t = 0) = 0 \quad \text{for } 0 < x < L \qquad [8]$$

where L (cm) is the length of the column.

The upper boundary condition of the soil column is a third-type boundary and is given as

$$\begin{cases} vC(x = 0,t) - v\lambda \dfrac{\partial C(x = 0,t)}{\partial x} = vC_0(t) \quad \text{for } 0 < t < t_0 \\ vC(x = 0,t) - v\lambda \dfrac{\partial C(x = 0,t)}{\partial x} = 0 \quad \text{for } t > t_0 \end{cases} \qquad [9]$$

and the lower boundary condition of the soil column is given as

$$\frac{\partial C(x = L,t)}{\partial x} = 0 \quad \text{for } t > t_0 \qquad [10]$$

where t_0 (h) is the pulse duration and $C_0(t)$ is the dimensional concentration from time zero to t_0.

The computer software HYDRUS-1D v3.0 (Šimůnek et al., 2005) was used to model the nonequilibrium transport of the testosterone miscible-displacement experiments. This program uses a nonlinear least-square routine to inversely model the experimental data and obtain optimized model parameters. The inverse routine iteratively fits a numerical solution of Eq. [7] to the observed data until an optimal fit is achieved. The optimum set of parameters is the one that provides the lowest sum-of-squared residuals (SSQ) between the observed data and modeled solution.

RESULTS AND DISCUSSION

Batch Experiments

The results of the batch experiment (Fig. 3) showed a rapid decrease in aqueous ^{14}C concentrations between 0 and 5 h, followed by a steady increase in ^{14}C for the duration of the experiment. Other batch sorption studies with hormones have reported a similar increase in aqueous concentrations (Casey et al., 2004; Lai et al., 2002) and have attributed it to the presence of metabolites in the aqueous phase. Thin layer chromatography could not be used to determine the metabolites of testosterone in the aqueous phase due to a lack of radioactivity left in the aqueous phase. However, various reasons for the increase in the aqueous concentration were surmised based on independent incubation experiments on this Hamar soil (Fan et al., 2007) and based on other literature. These reasons included desorption, mineralization, biodegradation, and/or photodegradation.

To examine whether desorption and photodegradation occurred, two parallel batch experiments in clear and amber vials with autoclaved soils were conducted. The results of these experiments are shown in Fig. 4, where the vertical scale is expanded to show that the difference between each experiment was small. In contrast to the batch experiments for natural soils (Fig. 3), the aqueous ^{14}C concentrations did not increase for the autoclaved or sterilized soils for the duration of the experiment in both the clear and amber vials (Fig. 4). Also the sorption isotherms from the clear vials were very similar to the isotherms of the amber vials. These results suggested that bioactivity (i.e., biodegradation and mineralization) was the major cause for the increase in aqueous ^{14}C concentrations in the batch experiments, not photodegradation or desorption (Fig. 3).

Several other studies tried to model testosterone batch studies using the sorbed and aqueous equilibrium concentrations (Casey et al., 2004; Mansell et al., 2004). However, it is difficult or impossible to reach true equilibrium in a short period of time during these types of experiments due to complex biodegradation, mineralization, and mass transfer between sorbed and aqueous phases. As a result, inaccuracies in parameter estimates and fate and transport mechanisms were likely for these previous studies. The approach of this study was to use nonequlibrium Eq. [1–3] to model these batch experiments for the native Hamar soil. The estimated parameters for the simultaneous transformation, kinetic

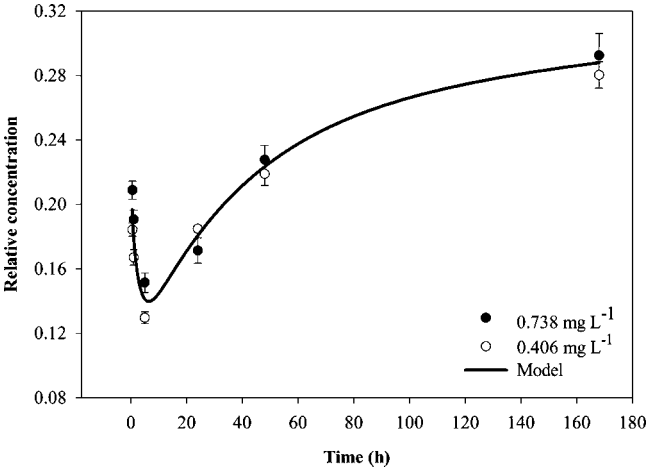

Fig. 3. Results of batch experiments for normal soils showing the aqueous concentration of ^{14}C through time. The relative concentration used in this figure and the other figures represents the ratio between the measured concentration and the initial concentration. The batch data shown are from the two different initial aqueous concentrations, which are fitted with the model described in Eq. [1], [2], and [4].

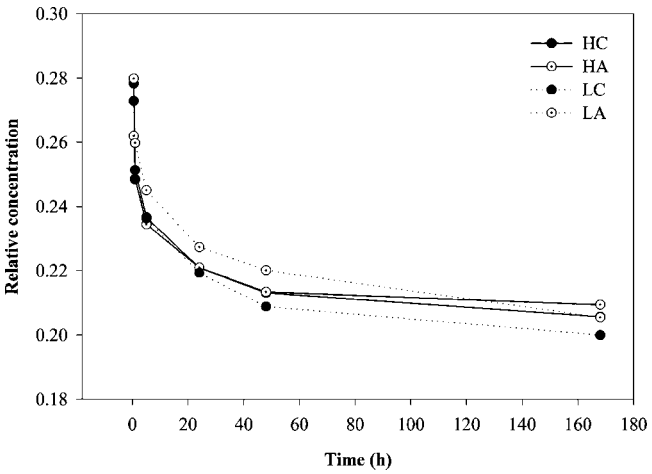

Fig. 4. Results of batch experiments for autoclaved soils showing the aqueous concentration of ^{14}C through time. The variables HC and HA represent initial aqueous concentration of 0.738 mg L^{-1} using clear and amber vials, respectively. The variables LC and LA represent initial aqueous concentration of 0.406 mg L^{-1} and clear and amber vials, respectively.

adsorption, and mineralization of testosterone and its metabolite are shown in Table 1. These equations assumed that the biodegradation of testosterone and its metabolite only occurred in the aqueous phase. This assumption was reasonable because organic contaminants, including nonaqueous phase liquids, solid compounds, and sorbed substrates, have to be dissolved or desorbed into the aqueous phase to be available for microbial consumption (Harms and Bosma, 1997). Additionally, microorganisms would reduce the concentration of testosterone in the aqueous phase by degradation and transformation, which would result in desorption of the sorbed testosterone back to the aqueous phase. Note that this desorption may be facilitated by biological rather than physical processes, and was expressed as a first-order mass exchange process (Eq. [1–3]) or α, which represents the mass transfer between aqueous and sorbed phase. Equations [1–3] also assumed that all of the $^{14}CO_2$ released during the experiments was dissolved in the aqueous phase. This assumption was also reasonable because even if all of the testosterone was mineralized to $^{14}CO_2$, then the concentration of $^{14}CO_2$ would be approximately 2.14×10^{-6} g mL^{-1}, which is much less than the solubility of CO_2 in water or 1.45×10^{-3} g mL^{-1} at 25°C and 1 atm (Weast, 1981). This assumption was also supported by the fact that the vials were sealed throughout the experiments, which prevented $^{14}CO_2$ from escaping. The results of the batch experiments (Fig. 3) showed that the relative aqueous concentration was independent of the initial concentration, which made it reasonable to assume that all of the degradation and mineralization rate constants could be expressed as first-order processes. Additionally, the global optimization routine assured the uniqueness of the estimated parameters (Moles et al., 2003), where the 95% confidence intervals of these estimates were small (Table 1). Finally, the confidence in these parameter estimates and in the accurate identification of the fate and transport mechanisms were high, because these parameters and the processes they described were used independently to describe the column experiments and provided excellent model fits to the data (presented latter).

Figure 5 shows the simulated concentrations of testosterone, its metabolite, and $^{14}CO_2$ in the aqueous and sorbed phase during the batch experiments. The aqueous concentrations of both testosterone and its metabolite (the unidentified polar metabolite) rapidly decreased at the beginning of the simulation and continued to gradually decrease throughout the duration of the simulation (Fig. 5A). In a corresponding manner, the sorbed testosterone concentrations continually decreased, while the sorbed concentration of the unidentified polar metabolite increased throughout the batch experiment simulation (Fig. 5B). The simulation also indicated that the most of the $^{14}CO_2$ production resulted from the mineralization of the unidentified polar metabolite, which has a lower sorption affinity (Fig. 5C). Some conclusions about the fate of testosterone through time in these batch experiments may be drawn from these real and simulated results. First, testosterone was quickly sorbed onto the soil particles. Second, the testos-

Table 1. The model parameter† estimates for batch and miscible-displacement experiments.

Parameter	v	λ	$K_{d,1}$	$\omega_{m,1}$	$\omega_{w,1}$	α_1	$K_{d,2}$	$\omega_{m,2}$	α_2	r^2	^{14}C recovery		
											Effluent	$^{14}CO_2$	Total
	cm h^{-1}	cm	L g^{-1}	h^{-1}			L g^{-1}	h^{-1}			%		
Batch experiments	NA‡	NA	0.289 (0.283–0.303)§	0.006 (0.004–0.008)	1.824 (1.813–1.966)	0.133 (0.132–0.140)	1.487 (1.042–1.540)	0.096 (0.093–0.099)	0.001 (0.0009–0.0014)	0.97	NA	NA	NA
Column experiments													
Cl^- (Experiment 1)	5.28	3.195 (3.193–3.197)	NA	NA	NA	NA	NA	NA	NA	0.99	NA	NA	NA
Testosterone (Experiment 1)	5.28	3.195	0.289	0.006	1.824	0.133	1.487	0.942 (0.928–0.964)	0.840 (0.674–1.006)	0.91	4.48	NA	47.81 (44.42–51.20)
Cl^- (Experiment 2)	5.04	5.296 (5.292–5.300)	NA	NA	NA	NA	NA	NA	NA	0.98	NA	NA	NA
Testosterone (Experiment 2)	5.04	5.296	0.289	0.006	1.824	0.133	1.487	0.942 (0.910–0.974)	0.840 (0.592–1.088)	0.84	13.25	23.4	79.75 (74.34–85.56)

† v, pore water velocity; λ, dispersivity; $K_{d,1}$, testosterone sorption distribution coefficient; $\omega_{m,1}$, testosterone mineralization rate constant; $\omega_{w,1}$, testosterone transformation rate constant; α_1, testosterone sorption mass-transfer coefficient; $K_{d,2}$, metabolite sorption distribution coefficient; $\omega_{m,2}$, metabolite mineralization rate constant; α_2, metabolite sorption mass-transfer coefficient; r^2, coefficient of determination.

‡ NA indicates that this parameter is not applicable to this particular model.

§ The value inside parentheses represents the 95% confidence interval, and also indicates that this parameter was estimated.

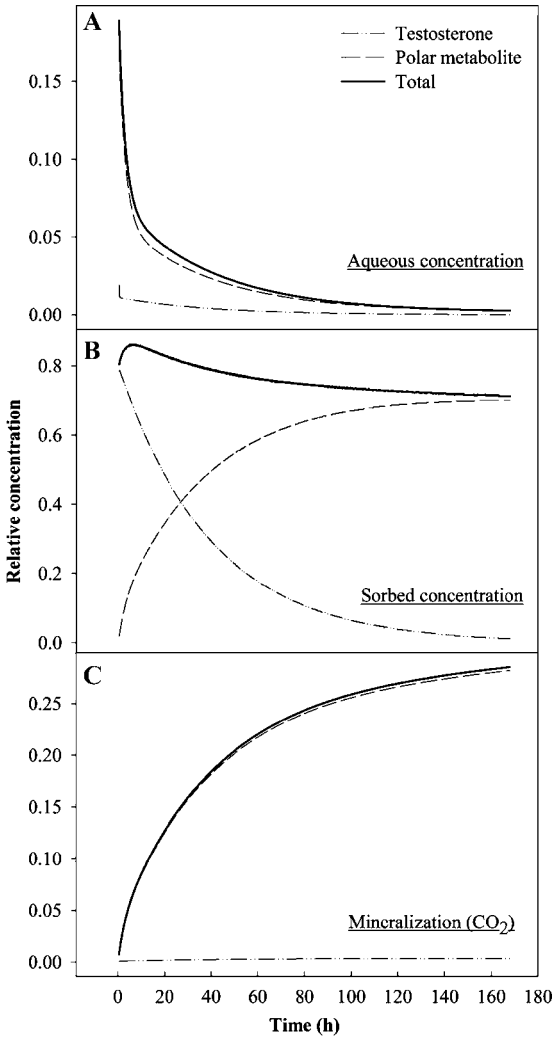

Fig. 5. Simulation of (A) aqueous concentrations of testosterone and its metabolite through time, (B) sorbed concentrations of testosterone and its metabolite through time, and (C) $^{14}CO_2$ created from testosterone mineralization through time. These simulations were calculated using the parameters obtained from fitting Eq. [1], [2], and [3] to the experimental batch data.

terone still present in the aqueous phase was quickly biodegraded to an unidentified polar metabolite and/or mineralized to $^{14}CO_2$. Third, the decrease of testosterone in the aqueous phase resulted in sorbed testosterone desorbing back into the aqueous phase, and then continually biodegrading to an unidentified polar metabolite. Finally, the unidentified polar metabolite was then sorbed onto the soil particles and/or mineralized to $^{14}CO_2$. As a result, all of the testosterone will biodegrade to the unidentified polar metabolite. Some of the degradation pathway of testosterone is still not fully evident from the present experiments or simulations. Specifically, it was not clear whether testosterone was directly degraded to the polar compound or whether another mechanism is involved in these degradation pathways. It can be noted that the high mineralization and degradation rates may be due to optimal geochemical conditions and high microbial activity, which are related to factors such as soil moisture and temperature (Lorenzen et al., 2004). Several other factors, such as organic matter (OM), amendment condition, and chemical properties of soil, also affected degradation and mineralization rates. Jacobsen et al. (2005) reported that the dissipation rate of testosterone was higher in the soil amended with high-OM manure than in the soil amended with low-OM manure. This may have been because high OM reduced the bioavailability of testosterone (Jacobsen et al., 2005).

Miscible-Displacement Experiments

The range of ^{14}C recovered (4.48–13.25%) from the column effluent from miscible-displacement experiments (Table 1) was low, which indicated that only small amounts of testosterone and its metabolite eluted from the soil columns. Soil combustion analyses revealed the vertical redistribution of the testosterone in the soil columns (Fig. 6), and indicated that most of the ^{14}C (70–74% of the mass recovered from inside the column) was sorbed in the top 1 to 5 cm for both experiments. Also, very little (0.6–1.74% of the mass recovered from inside the column) ^{14}C was found in the lower 5 cm of the column. These results were similar to other studies (Casey et al., 2004; Das et al., 2004).

Table 1 shows that the total mass recovery for Experiment 1 was only 47.81%, compared with 79.75% for Experiment 2. The results for Experiment 2 also showed that 23.4% of testosterone was mineralized to $^{14}CO_2$. The lower mass recovery for Experiment 1 compared with Experiment 2 was evidently caused by the emission of $^{14}CO_2$, which was not trapped in Experiment 1. However, the total mass recovery in Experiment 2 was still only 80%, which may have resulted from incomplete combustion. The TLC analysis on soil extracts from the soil within the column for Experiment 1 indicated that 28.4% of ^{14}C was metabolized to an unidentified polar metabolite, and only about 3.98% of ^{14}C was recovered as parent testosterone. In comparison, the TLC analysis on the soil extracts for Experiment 2, where the soil was sterilized at the end of the experiment, indicated that

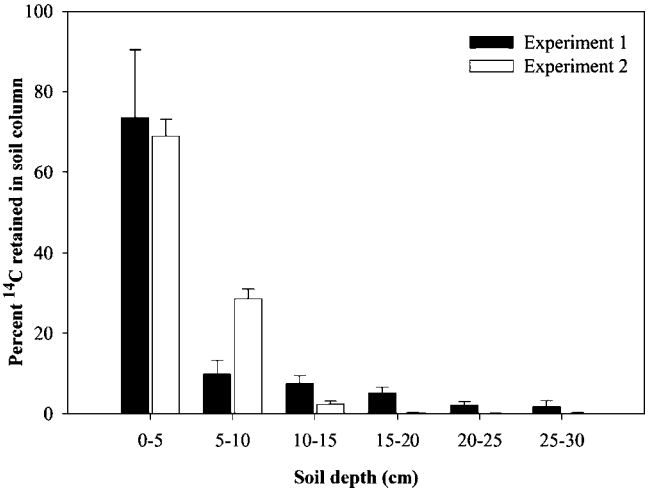

Fig. 6. The results of combustion experiment showing the distribution of ^{14}C with depth in the soil columns at the completion of the miscible-displacement Experiments 1 and 2.

about 50% of ^{14}C was recovered as parent testosterone, and only 7.07% of ^{14}C was found to be the unidentified polar metabolite. This indicated that testosterone continued to degrade and mineralize as the soil was extruded from the soil column in Experiment 1, and would further explain the lower mass recovery of Experiment 1 (i.e., the $^{14}CO_2$ would have been lost).

Despite differences in the mass balances of Experiment 1 and 2, their breakthrough curves were very similar (Fig. 7). The tailing that was observed for these breakthrough curves could be explained by either physical (e.g., preferential flow) and/or chemical (e.g., rate-limited sorption) nonequilibrium. The chloride breakthrough curves (Fig. 8), however, did not exhibit tailing or early solute breakthrough, which are indicative of physical nonequilibrium transport processes. Also, these chloride breakthrough curves were successfully described using an equilibrium convective-dispersive equation, i.e., Eq. [7] was used with $K_{d,1} = K_{d,2} = \omega_{w,1} = \omega_{m,1} = \omega_{m,2} = \alpha_1 = \alpha_2 = 0$. The excellent fit of the equilibrium model to the chloride ion breakthrough curves (Fig. 8; Table 1) further indicated that there was essentially no physical nonequilibrium transport. By deduction, the observed tailing for the testosterone breakthrough curves (Fig. 7) resulted from chemical and not physical nonequilibrium transport processes.

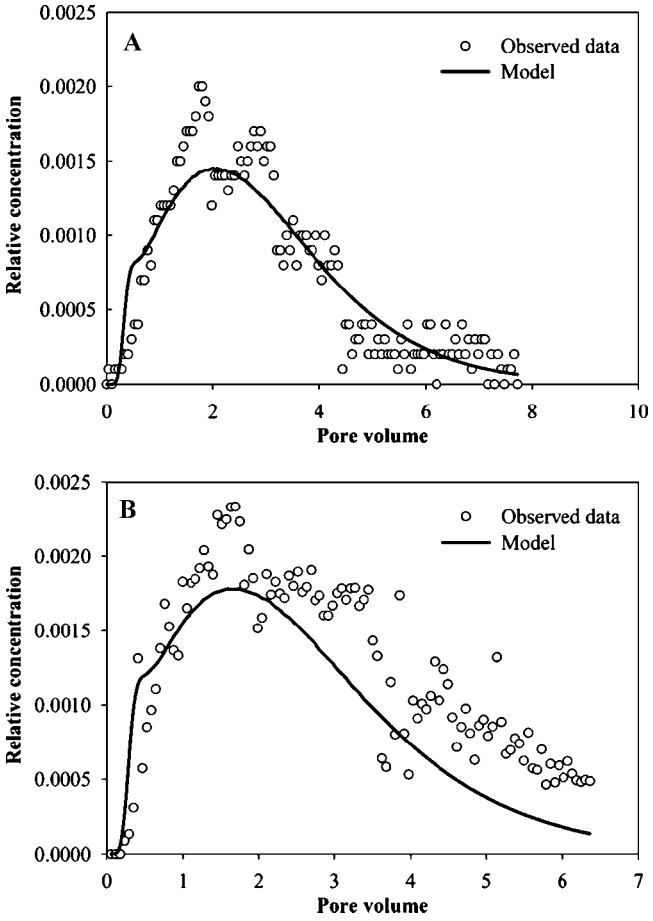

Fig. 7. Breakthrough curves of testosterone and its metabolite for miscible-displacement (A) Experiment 1 and (B) Experiment 2.

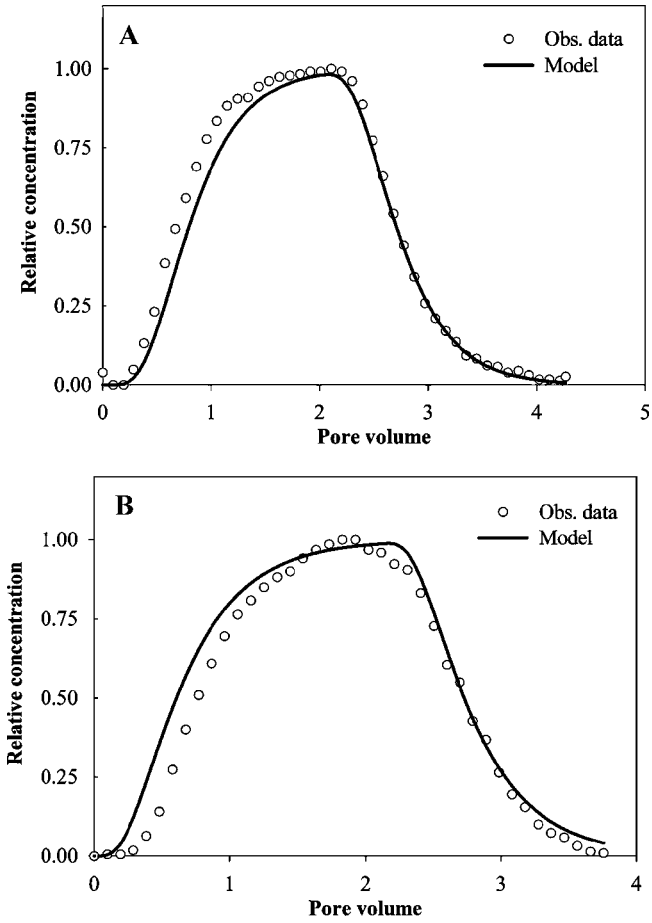

Fig. 8. Chloride ion breakthrough curves for miscible-displacement (A) Experiment 1 and (B) Experiment 2.

For the modeling of the testosterone breakthroughs, the $K_{d,1}$, $K_{d,2}$, $\omega_{w,1}$, $\omega_{m,1}$, and α_1 values in Eq. [7] were set equal to values obtained from the batch experiments and held constant. The testosterone breakthrough curves λ values were set equal to the λ values obtained from the chloride breakthrough curves and held constant. Additionally, the initial breakthrough curve model parameters, $\omega_{m,2}$ and α_2, were set equal to values obtained from the batch experiments and then optimized. Although a local optimization method was used in HYDRUS-1D to estimate these column parameters ($\omega_{m,1}$ and α_1) the confidence in these parameter estimates was high for several reasons. First, the testosterone and metabolite transport were optimized simultaneously, which improves the uniqueness of parameter estimates (Casey and Šimůnek, 2001). Second, the values obtained independently from the batch experiments, using global optimization, improved the uniqueness of the inverse model solution for the column experiments. Third, the number of parameters estimated was only two. Fourth, the estimated parameters agreed well with values obtained from other batch experiments and spanned realistic values.

The processes that the column transport model simulated provided accurate descriptions of the observed data. This result indicated that fate and transport processes, which the model simulated, were also accurate.

For example, Eq. [7] simulated that all of the detected ^{14}C in the column effluent would be metabolite, which meant that the testosterone transformed into a metabolite before it exited the soil column. This simulation was supported by the TLC analysis of the effluent that indicated that almost all of the ^{14}C in the column effluent was an unidentified polar metabolite.

The mass-transfer coefficient for the unidentified polar metabolite, α_2, was greater for the column experiments compared with the batch experiments (Table 1). The greater α_2 values indicate that the exchange between the aqueous and sorbed phase was faster in the soil column experiments (Zheng and Bennett, 2002). The reason for this might be that as this unidentified polar metabolite eluted from the soil column then it would cause more unidentified polar metabolite to desorb back into the aqueous phase to maintain the equilibrium between the aqueous and sorbed phases. In the batch experiments, this compound was retained in the aqueous phase through the entire duration of the experiments, which caused slower mass-transfer rates between the aqueous and sorbed phases. The mass transfer of the polar metabolite between the aqueous and sorbed phases can also explain the cause of the long eluting tail that was observed in these breakthrough curves. As mentioned above, the TLC analysis also indicated that nearly all of the ^{14}C in the effluents was recovered as the unidentified polar metabolite.

The mineralization rates of the unidentified polar metabolite, $\omega_{m,2}$, from the column experiments were greater than rates from the batch experiments. This may have been caused by differences in the dissolved oxygen concentrations in the two experimental systems, where mineralization rates of steroid hormones are related to the availability of dissolved oxygen (Colucci et al., 2001). In the column experiments, dissolved oxygen would have been continually introduced into the soil column with the influent water, thus maintaining high mineralization rates. The batch experiments, on the other hand, were closed systems where the oxygen would be consumed in biological processes and not replenished resulting in lower mineralization rates. For example, Fan et al. (2007) showed that the mineralization rate of testosterone under anaerobic conditions is much slower than under aerobic conditions.

CONCLUSIONS

The experiments and models developed in this article addressed various weakness associated with discerning hormone fate and transport in the environment. The correct identification of testosterone fate and transport processes and the accurate quantification of these processes were indicated by the consistency between the static (batch studies) and dynamic (column studies) experiments. Still, there are many uncertainties when applying these laboratory results to real environmental situations, where hormones are consistently detected (Kolpin et al., 2002; Herman and Mills, 2003). Consistent detections of these labile compounds could result from a lack of understanding of biologically mediated fate processes, which include bioavailability, temperature, redox conditions, moisture content, nutrient contents, etc. Moreover, little is known about natural background hormones concentrations in the environment (e.g., contributed from wildlife), and whether there is a steady-state level resulting from contributions from various natural and/or manmade sources.

ACKNOWLEDGMENTS

This research was based on work supported by the National Science Foundation under grant no. 0244169. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. The authors greatly appreciate the tremendous contributions made by Mrs. Barbara K. Magelky and Mrs. Colleen M. Pfaff (Biosciences Research Lab., USDA-ARS, Fargo, ND).

REFERENCES

Banga, J.R., E. Balsa-Canto, C.G. Moles, and A.A. Alonso. 2003. Dynamic optimization of bioreactors: A review. Proc. Indian Natl. Sci. Acad., Part A 69:257–265.

Casey, F.X.M., H. Hakk, J. Šimůnek, and G.L. Larsen. 2004. Fate and transport of testosterone in agricultural soils. Environ. Sci. Technol. 38:790–798.

Casey, F.X.M., and J. Šimůnek. 2001. Inverse analyses of transport of chlorinated hydrocarbons subject to sequential transformation reactions. J. Environ. Qual. 30:1354–1360.

Cohen, S.D., and A.C. Hindmarsh. 1994. CVODE user guide. CA Lawrence Livermore National Lab., Numerical Mathematics Group, Livermore, CA.

Colucci, M.S., H. Bork, and E. Topp. 2001. Persistence of estrogenic hormones in agricultural soils: I. 17β-estradiol and estrone. J. Environ. Qual. 30:2070–2076.

Das, B.S., L.S. Lee, P.S.C. Rao, and R.P. Hultgren. 2004. Sorption and degradation of steroid hormones in soils during transport column studies and model evaluation. Environ. Sci. Technol. 38:1460–1470.

Efron, B., and R.J. Tibshirani. 1993. An introduction to the bootstrap. Chapman and Hall, New York.

Fan, Z., F.X.M. Casey, G.L. Larsen, and H. Hakk. 2007. Persistence and fate of 17β-estradiol and testosterone in agricultural soils. Chemosphere 67:886–895.

Harms, H., and T.N.P. Bosma. 1997. Mass transfer limitation of microbial growth and pollutant degradation. J. Ind. Microbiol. 18: 97–105.

Herman, J.S., and A.L. Mills. 2003. Biological and hydrogeological interactions affect the persistence of 17β-estradiol in an agricultural watershed. Geobiology 1:141–151.

Iguchi, T., H. Watanabe, and Y. Katsu. 2001. Developmental effects of estrogenic agents on mice, fish, and frogs: A mini-review. Horm. Behav. 40:248–251.

Jacobsen, A., A. Lorenzen, R. Chapman, and E. Topp. 2005. Persistence of testosterone and 17β-estradiol in soils receiving swine manure or municipal biosolids. J. Environ. Qual. 34:861–871.

Ji, X., and Y. Xu. 2006. libSRES: A C library for stochastic ranking evolution strategy for parameter estimation. Bioinformatics 22: 124–126.

Koger, C.S., S.J. Teh, and D.E. Hinton. 2000. Determining the sensitive developmental stages of intersex induction in medaka (*Oryzias latipes*) exposed to 17β-estradiol or testosterone. Mar. Environ. Res. 50:201–206.

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, and H.T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. Environ. Sci. Technol. 36: 1202–1211.

Lai, K.M., M.D. Scrimshaw, and J.N. Lester. 2002. Prediction of the bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems. Sci. Total Environ. 289:159–168.

Larsson, D.G.J., H. Hällman, and L. Förlin. 2000. More male fish embryos near a pulp mill. Environ. Toxicol. Chem. 19:2911–2917.
Lee, L.S., T.J. Strock, A.K. Sarmah, and P.S.C. Rao. 2003. Sorption and dissipation of testosterone, and estrogens, and their primary transformation products in soils and sediments. Environ. Sci. Technol. 37:4098–4105.
Lintelmann, J., A. Katayama, N. Kurihara, L. Shore, and A. Wenzel. 2003. Endocrine disruptors in the environment. Pure Appl. Chem. 75:631–681.
Lorenzen, A., J. Hendel, K.L. Conn, S. Bittman, A. Kwabiah, G. Lazarovitz, D. Massé, T.A. McAllister, and E. Topp. 2004. Survey of hormone activities in municipal biosolids and animal manures. Environ. Toxicol. 19:216–225.
Mansell, J., J.E. Drewes, and T. Rauch. 2004. Removal mechanisms of endocrine disrupting compounds (steroids) during soil aquifer treatment. Water Sci. Technol. 50:229–237.
Moles, C.G., P. Mendes, and J.R. Banga. 2003. Parameter estimation in biochemical pathways: A comparison of global optimization methods. Genome Res. 13:2467–2474.
Panter, G.H., R.S. Thompson, and J.P. Sumpter. 1998. Adverse reproductive effects in male fathead minnows (Pimephales promelas) exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. Aquat. Toxicol. 42:243–253.
Runarsson, T.P., and X. Yao. 2000. Stochastic ranking for constrained evolutionary optimization. IEEE Trans. Evol. Comput. 4:284–294.
Shore, L.S., and M. Shemesh. 2003. Naturally produced steroid hormones and their release into the environment. Pure Appl. Chem. 75:1859–1871.
Šimůnek, J., M.T. van Genuchten, and M. Šejna. 2005. The HYDRUS-1D software package for simulating the one-dimensional movement of water, heat, and multiple solutes in variably-saturated media, Version 3.0. Dep. of Environ. Sci., Univ. of California, Riverside, CA.
van Genuchten, M.T. 1981. Non-equilibrium transport parameters from miscible displacement experiments. Research report No. 119. U.S. Salinity Laboratory, USDA-ARS, Riverside, CA.
van Genuchten, M.T. 1985. Convective-dispersive transport of solutes involved in sequential first-order reactions. Comput. Geosci. 11: 129–147.
Weast, R.C.E. 1981. CRC handbook of chemistry and physics (61st Edition). CRC Press, Inc., Boca Raton, FL.
Zheng, D.R., and G.D. Bennett. 2002. Applied contaminant transport modeling. 2nd ed. John Wiley & Sons, New York.